8. PHARMACOKINETIC MODELING

8.1. INTRODUCTION

Several physiologically based pharmacokinetic (PBPK) models of 1,3-butadiene metabolism and disposition have been developed to attempt to explain the interspecies differences in the potency and site specificity of the carcinogenic response between mice and rats and to provide a corresponding dosimetric basis for quantitatively extrapolating carcinogenic potency from rodents to humans (Hattis and Wasson, 1987; Hallenbeck, 1992; Kohn and Melnick, 1993; Johanson and Filser, 1993; Evelo et al., 1993; Medinsky et al., 1994). PBPK models use species-specific physiological parameters such as alveolar ventilation rates and blood flow rates, chemical-specific distribution parameters such as blood:air and tissue:blood partition coefficients, and species- and chemical-specific metabolic rates to elucidate the pharmacokinetics (i.e., the uptake, distribution, metabolism, and excretion) of a chemical.

Ideally, such models provide species-specific target tissue doses of the toxicologically active form(s) of the chemical. Carcinogenic risks from bioassay data can then be extrapolated to humans on the basis of equivalent effective doses, reducing some of the uncertainties that occur when interspecies extrapolation is based simply on exposure to the parent compound, especially when nonlinear physiological processes are involved. Assumptions must still be made to the effect that the mechanisms of action of the active form(s) of the compound at the target tissue(s) are the same across species and that the tissues of different species are equally sensitive. If these assumptions are not valid, pharmacodynamic data and modeling are required for more precise risk assessment.

PBPK models that fall short of describing target tissue doses of the active form(s) of a chemical may still be useful for improving the dosimetric basis of interspecies extrapolation for quantitative risk assessment. For example, it is well established that metabolic activation of 1,3-butadiene is probably necessary for its carcinogenic action (Chapter 4). Therefore, a PBPK model describing the production and disposition of 1,2-epoxy-3-butene (EB), the first product of metabolic activation of 1,3-butadiene, may be able to provide a better dose metric than the default methodology of using exposure to 1,3-butadiene itself.

This chapter reviews and analyzes the six PBPK models for 1,3-butadiene that are currently available and assesses their usefulness for quantitative risk assessment of 1,3-butadiene based on interspecies extrapolation. Each of these PBPK models assumes, for simplicity, that the transfer of 1,3-butadiene to tissues is blood flow-limited, that each tissue compartment is "well mixed," and that tissue concentrations are in equilibrium with the venous blood concentration leaving the tissue.

8.2. PBPK MODELS FOR 1,3-BUTADIENE

8.2.1. Hattis and Wasson (1987)

The first PBPK model for 1,3-butadiene was that of Hattis and Wasson (1987). They defined the effective dose of 1,3-butadiene as the amount that is metabolically converted to EB and used this dose as a basis for a risk assessment of occupational 1,3-butadiene exposure. Their model consists of three compartments: a fat compartment; a muscle compartment; and a liver and vessel-rich compartment, which includes the brain, heart, kidneys, and other small visceral organs. The transfer of 1,3-butadiene between blood and tissues is assumed to be blood flow-limited. Metabolism to the monoepoxide is ascribed to the entire liver and vessel-rich compartment and is assumed to follow simple Michaelis-Menten kinetics. No further metabolism of EB is considered.

The only chemical-specific parameter values then available were whole-body maximal metabolic rates for mice and rats inferred from the chamber study data of Kreiling et al. (1986b). These data provided the K_M and preliminary V_{max} estimates for the liver and vessel-rich compartment. Tissue:blood and blood:air partition coefficients were estimated from chemical structure and solubility data using empirical relationships (e.g., Fiserova-Bergerova and Diaz, 1986). Model simulations were then run, adjusting K_M and the partition coefficients to fit the blood 1,3-butadiene concentration data of Bond et al. (1986), to derive "best estimates" for these parameters. Human metabolic rates were estimated by allometric scaling of the mouse and rat rates because no PBPK data were available for human metabolism of 1,3-butadiene. The parameter values used by Hattis and Wasson (1987) are summarized in Table 8-1.

No additional data were available at that time for an independent validation of this model. A minimal sensitivity analysis was conducted by varying K_M and the blood:air partition coefficient among a few values and observing the effect on the ultimate risk estimates. Hattis and Wasson (1987) claimed that their model is not very sensitive to reasonable differences in partition coefficients. Similarly, the model is insensitive to the precise value of the metabolic parameters because, given the blood:air partition coefficient values that were used, metabolic conversion in their model is limited by blood flow to the liver and vessel-rich compartment. Hattis and Wasson concluded that differences in pharmacokinetics fail to account for differences in carcinogenesis between mice and rats and that, with respect to risk assessment, uncertainties in the PBPK modeling are trivial compared with the differences in apparent sensitivities between these species.

Table 8-1. Parameter values used in the Hattis and Wasson (1987) PBPK model

Parameter	Rat	Mouse	Human
Alveolar ventilation (L/min)	0.15	0.0233	11.38[a] 4.8
Weight (kg)	0.40	0.028	70
Q_f (L/min)	0.0136	0.00192	0.69[a] 0.35[b]
Q_m (L/min)	0.0226	0.00319	2.61[a] 1.1[b]
Q_{lvr} (L/min)	0.1042	0.01617	5.09[a] 4.35[b]
V_f (L)	0.028	0.0028	14.024
V_m (L)	0.300	0.0196	34.756
V_{lvr} (L)	0.036	0.00308	8.513
Blood:air partition coefficient[c]	---------- 0.35 ----------		
P_f	---------- 118.2 ----------		
P_m	---------- 5.26 ----------		
P_{lvr}	---------- 5.4 ----------		
V_{max} (mol/min)	1.47E-6[d]	1.87E-7[d]	8.0E-5[e]
K_M (mol/L)	---------- 5E-6 [f] ----------		

[a]Awake.
[b]Asleep.
[c]The blood:air partition coefficient of 0.35 is the "best estimate" value from "fitting" the model. The tissue:blood partition coefficients (P) are from functions of the blood:air partition coefficient for which the "best estimate" value of 0.35 was used. Partition coefficients are assumed to be the same across species.
[d]From Kreiling et al. (1986b).
[e]From allometric scaling of the rodent values.
[f]"Best estimate" from "fitting" the model.

Subscripts f, m, and lvr designate the fat, muscle, and liver and vessel-rich compartments (tissues), respectively.
Q: tissue blood flow rate.
V: tissue volume.
P: tissue:blood partition coefficient.

The Hattis and Wasson (1987) model is not discussed further here because it has been superseded by new data and other modeling efforts.

8.2.2. Hallenbeck (1992)

Hallenbeck (1992) reported having done a PBPK-based cancer risk assessment for 1,3-butadiene; however, he provided no details of the PBPK model that he used. Furthermore, he used the area under the 1,3-butadiene concentration-versus-time curve for the lung as his tissue-dose surrogate, taking no account of metabolic activation. As presented, this model contributes nothing to the current state of knowledge regarding the pharmacokinetic modeling of 1,3-butadiene.

8.2.3. Kohn and Melnick (1993)

The PBPK model of Kohn and Melnick (1993) focuses on the disposition of EB in the mouse, rat, and human. This model incorporates additional tissues (compartments) and metabolic reactions based on experimental data that were not available at the time of the Hattis and Wasson (1987) model; however, it also relies on theoretically derived partition coefficients. The Kohn and Melnick model is blood flow-limited and consists of six compartments: lung, blood, fat, liver, other rapidly perfused tissues (viscera), and slowly perfused tissues (muscle). Metabolism occurs in the liver, lung, and viscera compartments. The metabolic reactions include conversion of 1,3-butadiene to EB, the conversion of EB to 1,2:3,4-diepoxybutane (DEB), the enzymatic hydrolysis of EB, and the enzymatic conjugation of EB with glutathione.

With the exception of the partition coefficients, which were derived in advance from published methodologies, all of the mouse, rat, and human parameter estimates were from the literature; none of them were adjusted to obtain a fit to experimental data. The parameter values used by Kohn and Melnick (1993) are summarized in Table 8-2. Blood:tissue partition coefficients for 1,3-butadiene were from Hattis and Wasson (1987). The blood:air partition coefficients reported by Csanády et al. (1992) for 1,3-butadiene and EB were used as lung:air partition coefficients. The fat:blood partition coefficient for EB was calculated using an empirical relationship from Lyman et al. (1990), whereas the tissue:blood partition coefficients of EB for the other tissues were derived using the method of Fiserova-Bergerova and Diaz (1986). These are essentially the same procedures used by Hattis and Wasson (1987).

Michaelis-Menten kinetics were used to describe the oxidation of 1,3-butadiene and EB by the cytochrome P-450 isozyme CYP2E1, the hydrolysis of EB by epoxide hydrolase, and the glutathione S-transferase-catalyzed conjugation of EB with glutathione. K_M and V_{max} values for each of these reactions in the liver and lung of the mouse, rat, and human were taken from the in

Table 8-2. Parameter values used in the Kohn and Melnick (1993) PBPK model

Parameter	Mouse	Rat	Human
Physiological parameters [a]			
Body weight (kg)	0.028	0.4	70
Cardiac output (L/h)	1.044	7.32	660[b]
Ventilation rate (L/h)	2.64	15.6	1,200[b]
Fraction blood	0.05	0.054	0.077
Fraction fat	0.04	0.08	0.144
Fraction liver	0.062	0.05	0.025
Fraction viscera	0.05	0.083	0.037
Fraction muscle	0.78	0.59	0.547
Fat flow fraction	0.05	0.07	0.036
Liver flow fraction	0.16	0.16	0.16
Viscera flow fraction	0.52	0.40	0.446
Muscle flow fraction	0.19	0.36	0.361
Partition coefficients [c]			
Air partition BD		1.5	
Fat partition BD		118.2	
Liver partition BD		5.49	
Viscera partition BD		5.34	
Muscle partition BD		5.26	
Air partition EB		60	
Fat partition EB		1.8083	
Liver partition EB		0.6545	
Viscera partition EB		0.6348	
Muscle partition EB		0.6533	

Table 8-2. Parameter values used in the Kohn and Melnick (1993) PBPK model (continued)

Parameter	Mouse	Rat	Human
Biochemical parameters[d]			
Liver V cyt1 (nmol/h/mg)	155.4	35.4	70.8
Liver Km cyt1 (mM)	0.002	0.00375	0.00514
Liver V cyt2 (nmol/h/mg)	12		
Liver Km cyt2 (mM)	0.0156		
Liver V EH (nmol/h/mg)	347.4	148.8	1,110
Liver Km EH (mM)	1.59	0.26	0.58
Liver V GST (nmol/h/mg)	30,000	14,460	2,706
Liver Km GST (mM)	35.3	13.8	10.4
Liver micro prot (mg/L)	11,600	16,800	14,500
Liver cyto prot (mg/L)	82,800	108,000	58,000
Lung V cyt1 (nmol/h/mg)	138.6	9.6	9
Lung Km cyt1 (mM)	0.00501	0.00775	0.002
Lung k hydr (h^{-1}/mg)	0.1116	0.0792	0.1914
Lung V GST (nmol/h/mg)	6,380	2,652	
Lung Km GST (mM)	36.5	17.4	
Lung k GST (h^{-1}/mg)			0.1536
Lung micro prot (mg/L)	3,000	3,000	3,000
Lung cyto prot (mg/L)	82,800	108,000	58,000

[a]Compartment volumes are given as fractions of body weight; compartment blood flow rates are given as fractions of cardiac output.
[b]Human cardiac output at rest: 336 L/h; human ventilation rate at rest: 240 L/h.
[c]Lung:air and tissue:blood; assumed same for all species.
[d]Data from Csanády et al. (1992).

BD: 1,3-butadiene; EB: 1,2-epoxy-3-butene.
V: V_{max}; Km: K_M.
cyt1 denotes oxidative metabolism of butadiene to EB; cyt2 denotes oxidative metabolism of EB.
EH: epoxide hydrolase.
GST: glutathione S-transferase.
micro prot: microsomal protein; cyto prot: cytoplasmic protein.
k hydr: apparent first-order rate constant for EB hydrolysis; k gst: apparent first-order rate constant for glutathione conjugation.

vitro data of Csanády et al. (1992). The lung values were also assumed to apply to the viscera compartment. Csanády et al. detected DEB formation only in mouse liver preparations. Therefore, Kohn and Melnick (1993) included this reaction only in the mouse liver compartment and only as a disappearance route for EB; the distribution of DEB was not further modeled. 1,3-butadiene and EB were treated as competitive inhibitors of each other in the rate equations for mouse liver CYP2E1. Finally, although glutathione was treated as saturating for glutathione S-transferase in the mouse, rat, and human liver, glutathione conjugation with EB in human lung and viscera was assumed to be first order.

To validate their model, Kohn and Melnick (1993) compared predicted 1,3-butadiene absorption and blood concentrations for mice and rats with the measurements of Bond et al. (1986). They also modified the model to include a chamber compartment and compared predicted EB concentrations in the chamber and maximum metabolic elimination rates with the Laib et al. (1990) results for mice and rats. Kohn and Melnick claimed that their model predictions are comparable to the experimental results except for overestimates in the blood 1,3-butadiene concentrations, which they ascribed to inadequacies in the model or experimental sources of error in the blood concentration measurements.

To assess the sensitivity of the model to the values of various parameters, relative sensitivity coefficients for different model variables were estimated by finite differences, as given by Frank (1978). The physiological parameters to which the model was the most sensitive were the lung:air partition coefficient and the cardiac output. Because the ventilation rate is greater than the rate of 1,3-butadiene absorption, the lung:air partition coefficient and the cardiac output are the major parameters governing 1,3-butadiene uptake. Predicted 1,3-butadiene concentrations were not very sensitive to variations in the biochemical parameters; however, monoepoxide levels were somewhat more sensitive to the parameters describing hepatic glutathione S-transferase and epoxide hydrolase kinetics.

Based on their model simulations, Kohn and Melnick (1993) reported that 1,3-butadiene uptake and the disposition of EB are controlled to a greater extent by physiological parameters than by biochemical parameters. The model further suggests that storage in fat is a significant fraction of retained 1,3-butadiene, especially in rats and humans. Kohn and Melnick also found that predicted EB tissue concentrations do not correlate with tumor incidences in mice and rats, and they concluded that other factors are crucial in 1,3-butadiene-induced carcinogenesis. These other factors may include pharmacokinetic variables that were not part of the model, such as accumulation of the diepoxide or formation of other metabolites or mechanistic (pharmacodynamic) phenomena, such as formation of DNA adducts or efficiency of DNA repair.

The Kohn and Melnick (1993) model appears to have a reasonable basic structure, in terms of the compartments and metabolic reactions included, given the biochemical parameters that are currently available. A major strength of their model is that none of the parameter estimates is adjusted to fit experimental data. Two important drawbacks of the model are the use of empirically derived partition coefficients and the lumping of various tissues with different metabolic capabilities (Chapter 3) into a viscera compartment, which is assumed to have the same metabolic activity as the lung. Partition coefficients for 1,3-butadiene and EB have recently been measured by Johanson and Filser (1993) and Medinsky et al. (1994), and experimental values for the 1,3-butadiene partition coefficients are substantially less than the empirically derived estimates, which suggests that the specific results reported by Kohn and Melnick may not be relevant. For example, the role of physiological parameters in controlling 1,3-butadiene uptake and the amount of 1,3-butadiene storage in fat may not, in fact, be as great as the Kohn and Melnick model predicts (Medinsky et al., 1994).

8.2.4. Johanson and Filser (1993)

Johanson and Filser (1993) developed a PBPK model for 1,3-butadiene and EB disposition in rats and mice. Their model is blood flow-limited and consists of four main physiological compartments**C**lungs and arterial blood, muscle and vessel-rich tissues, fat, and liver**C**as well as a chamber compartment and an intrahepatic subcompartment. Metabolism is assumed to take place exclusively in the liver. The metabolic reactions include oxidation of 1,3-butadiene to EB; hydrolysis of EB; intrahepatic first-pass hydrolysis of EB; conjugation of EB with glutathione, which is described by a "ping-pong" mechanism; and the turnover and depletion of hepatic glutathione.

In contrast with the previous PBPK modeling efforts for 1,3-butadiene, Johanson and Filser (1993) conducted in vitro studies of rat homogenates to obtain empirical values for the tissue:air partition coefficients for 1,3-butadiene and EB. All physiological parameters were taken from Arms and Travis (1988), except the alveolar ventilation rates, which were reduced to 60% of those suggested by Arms and Travis on the basis of generalized observations of uptake rates of various gases in closed-chamber experiments (Johanson and Filser, 1992). For the oxidative metabolism of 1,3-butadiene, the model uses the V_{max} values from the in vitro studies of Filser et al. (1992). A K_M value was derived by fitting the model to the in vivo data of Lieser (1983) for the rat and Kreiling (1986b) for the mouse because the model could not reproduce the results observed in these closed-chamber studies the K_M values of either Filser et al. (1992) or Csanády et al. (1992). Values for the metabolic parameters pertaining to the conjugation of EB with glutathione and to the hydrolysis of EB were taken from the in vitro data of Kreuzer et al. (1991). The value of the "intrinsic K_M" for the intrahepatic hydrolysis of EB (see below) was set

to 20% of the "apparent K_M" value of Kreuzer et al. because the model then fit various in vivo data. The flow rate between the hepatic and intrahepatic compartments was estimated from the kinetic parameters. The physiological and biochemical parameter values used by Johanson and Filser (1993) are summarized in Table 8-3.

In terms of the metabolic reactions involved, the Johanson and Filser (1993) model differs from the Kohn and Melnick (1993) model in that further oxidation of EB to DEB is not included, conjugation of EB with glutathione is described by the two-substrate ordered sequential ping-pong mechanism (reviewed by Mannervik, 1985) rather than by Michaelis-Menten kinetics, and glutathione turnover and the intrahepatic first-pass hydrolysis of EB are incorporated. Given the K_M values for glutathione conjugation used in the model, the conjugation of EB becomes rate-limited by glutathione only when glutathione is almost completely depleted. Cytosolic glutathione turnover is depicted by zero-order production and first-order elimination. Intrahepatic first-pass hydrolysis of EB is hypothesized to occur, based on the observations of Filser and Bolt (1984), because of proximity of the monooxygenase to the epoxide hydrolase in the endoplasmic reticulum. Newly formed EB within this intrahepatic compartment will be more readily hydrolyzed than EB that must diffuse in from outside the compartment, as reflected by a lower K_M in the intrahepatic compartment.

To attempt to validate the model, Johanson and Filser (1993) compared simulated results with the data from various in vivo experiments. In addition to the 1,3-butadiene kinetics data used to fit the K_M for 1,3-butadiene oxidation and the EB kinetics data of Filser and Bolt (1984) for the rat and Kreiling (1987) for the mouse that were used to fit the intrinsic K_M for intrahepatic first-pass hydrolysis, the model apparently reproduces the EB concentrations appearing in chamber air as a result of 1,3-butadiene exposure in the experiments of Rolzhäuser (1985) for the rat and Kreiling (1987) for the mouse. However, it is not clear from the text whether these experimental data were also used to fit the intrinsic K_M. The model also reproduces the glutathione concentrations observed by Deutschmann (1988) in rat and mouse liver after 1,3-butadiene exposure, and Johanson and Filser claimed that no model parameters were fitted to these data. Finally, simulated blood concentrations of EB approximate those observed by Bond et al. (1986) in the mouse but are slightly higher than those observed in the rat.

No sensitivity analysis for the model parameters was reported.

The results of Johanson and Filser's (1993) model simulations suggest that the internal dose of EB, expressed as the concentration of EB or the area under the concentration-time curve

Table 8-3. Parameter values used in the Johanson and Filser (1993) PBPK model

Parameter		Mouse	Rat
Physiological data			
Body weight (g)	Standard animal	25	250
	Simulations	27.5	157.5-217.5[a]
Alveolar ventilation (mL/min)	Standard animal	15	70.2
	Simulations	proportional to $bw^{2/3}$	
Cardiac output (mL/min)	Standard animal	17	83
	Simulations	proportional to $bw^{2/3}$	
Blood flows (% of cardiac output)	Muscle and VRG	66	66
	Fat	9	9
	Liver	25	25
Compartment volumes (% of body weight)	Lung and arterial	1	1
	Muscle and VRG	75	80
	Fat	10	7
	Liver	5.5	4
Partition coefficients [b]			
1,3-Butadiene	Lung and arterial, muscle and VRG, liver	0.25	
	Fat	7.23	
	Blood	3.03	
1,2-Epoxy-3-butene	Lung and arterial, muscle and VRG, liver	0.706	
	Fat	1.89	
	Blood	83.4	

Table 8-3. Parameter values used in the Johanson and Filser (1993) PBPK model (continued)

Parameter		Mouse	Rat
Metabolic constants			
1,3-Butadiene oxidation	Microsomal protein (mg/g liver)	30	30
	V_{max} (nmol·min^{-1}·mg^{-1})[c]	3.22	2.17
	K_M (µmol/L air)[d]	5	5
EB hydrolysis	Microsomal protein (mg/g liver)	30	30
	V_{max} (nmol·min^{-1}·mg^{-1})[e]	19	17
	Apparent K_M (mmol/L)[e]	1.5	0.7
	Intrinsic K_M (% of apparent K_M)[d]	20%	20%
EB conjugation	Cytosolic protein (mg/g liver)	95	95
	V_{max}/K_M of EB (µL·min^{-1}·mg^{-1})[e]	15	11
	K_M toward EB (mmol/L)[e]	100	100
	K_M toward glutathione (mmol/L)[f]	0.1	0.1
Glutathione kinetics	Initial steady-state concentration (mmol/L)	8.31[g] 5.5[h]	5.56[g] 4.2[h]
	Elimination rate constant (h^{-1})[f]	0.15	0.15

[a]Depending on experiment simulated.
[b]Tissue:blood and blood:air; assumed same for all species.
[c]From Filser (1992).
[d]Obtained by best fit.
[e]Kreuzer et al. (1991).
[f]Average of literature data.
[g]Deutschmann and Laib (1989).
[h]Kreiling et al. (1988).

VRG: vessel-rich tissue group.
EB: 1,2-epoxy-3-butene.
bw^b = (body weight)^b.

in the venous blood, the other compartments, or the whole body, is at most about three times greater in the mouse than in the rat for a given exposure concentration. The greatest differences in internal dose of EB between the two species result from 1,3-butadiene exposure concentrations of above 1,000 ppm, when glutathione depletion occurs in the mouse but not in the rat after 6 to 9 h of exposure. Once again, the relatively small interspecies differences in body burden of EB indicated by PBPK modeling cannot explain the striking differences in cancer response between mice and rats exposed to 1,3-butadiene. Johanson and Filser suggested that differences in the kinetics of DEB or nonmetabolic factors, such as differences in immune response or in the expression of oncogenes, may be responsible for the interspecies differences in cancer response.

A major advancement found in the PBPK model of Johanson and Filser (1993) is the use of experimentally derived partition coefficients, especially because these values differ substantially from the theoretically estimated values. A further strength of their analysis is that they compared the simulation results with data from several different experiments. The Johanson and Filser model also incorporates hepatic glutathione turnover and depletion as well as intrahepatic first-pass hydrolysis of EB, although the significance of these refinements is unknown. Some of the limitations of the model include the exclusion of extrahepatic metabolism and of further metabolism of EB to DEB. In addition, the values of the K_M for 1,3-butadiene oxidation and of the intrinsic K_M for intrahepatic first-pass hydrolysis of EB were obtained by fitting in vivo data. Finally, no sensitivity analysis was reported, although, for example, it was acknowledged that wide ranges of glutathione concentrations and turnover rates have been observed. Therefore, it is unknown how sensitive the model is to changes in these and other parameters. Johanson and Filser are reportedly working on a corresponding PBPK model for humans, but it has not yet been published.

8.2.5. Evelo et al. (1993)

Evelo et al. (1993) present a PBPK model for the uptake, distribution, and metabolic clearance of 1,3-butadiene in mice and rats. Their stated objective was to investigate the relative importance of liver and lung metabolism at different 1,3-butadiene exposure concentrations. The Evelo et al. model has six physiological compartments: liver, fat, muscle, a vessel-rich group, the bronchial area of the lung, and the alveolar area of the lung. A chamber compartment is also included for validation against the data from closed-chamber experiments. 1,3-Butadiene metabolism is assigned to both the alveolar and bronchial areas of the lung and to the liver. Gas exchange occurs in the alveolar area of the lung.

Values for the standard physiological parameters were allometrically scaled from the data of Travis (1988). Volumes and blood flows for the two separate lung compartments were taken

from Greep and Weis (1977). Tissue:blood and blood:air partition coefficients were theoretically estimated using the regression analysis method of Fiserova-Bergerova and Diaz (1986), as was done previously by Hattis and Wasson (1987).

To describe the oxidation of 1,3-butadiene to EB, Evelo et al. (1993) calculated the ratios of the maximum metabolic activity between the liver and the lung from the in vitro data of Schmidt and Loesser (1985) for the mouse and for the rat. Then, the total (whole-body) maximum metabolic activities, the K_Ms, and "the most probable distribution" of metabolic activity between the alveolar and bronchial areas of the lung were derived by optimizing the model against the closed-chamber data of Kreiling et al. (1986b) for the mouse and Bolt et al. (1984) for the rat. The only options considered for the distribution of the metabolic activity of the lung were that all the metabolism took place in either one of the two areas, that it was equal in each area, or that it was distributed relative to the volumes of each area; the best fit was found using the latter distribution. The values of the physiological and metabolic parameters used in the Evelo et al. model are summarized in Table 8-4.

The only independent validation of the model was against the whole-body extraction ratios reported by Dahl et al. (1990). Evelo et al. (1993) calculated extraction ratios of 8.4% for the mouse and 5.2% for the rat, whereas Dahl et al. found ratios of 12.8% for the mouse and 4.3% for the rat. Evelo et al. also noted that the whole-body V_{max} value obtained for the rat by fitting the model to the data of Bolt et al. (1984) does not fall within the range of values allowed by experimental error based on the gas-uptake studies of Laib et al. (1992).

Evelo et al. (1993) stated that sensitivity analyses found the model optimization to be relatively insensitive to variability in the value of K_M. No other sensitivity analysis results are reported.

The model simulations of Evelo et al. (1993) suggest that the relative importance of 1,3-butadiene metabolism in the mouse lung is greater than the distribution of metabolic activity would imply, especially at exposure concentrations of less than 200 ppm and for K_M values of less than the "best fit" value. Evelo et al. concluded that there is a strong first-pass effect in the mouse lung. At higher concentrations, alveolar metabolism is saturated, and liver metabolism becomes relatively more important. The relative importance of lung metabolism also increases with decreasing exposure concentration for the rat and human, especially with lower values of K_M; however, unlike for the mouse, the lung metabolism never exceeds the liver metabolism. Evelo et al. suggested that the higher rate of metabolic activation in the mouse lung could be responsible for the mouse's greater sensitivity to developing lung carcinomas and heart hemangiosarcomas from exposure to 1,3-butadiene.

Table 8-4. Parameter values used in the Evelo et al. (1993) PBPK model

Parameter	Mice	Rats
Physiological parameters		
Body mass (kg)	0.0275	0.215
Cardiac output (mL/min)	24.83	75.93
Alveolar ventilation (mL/min)	24.5	118.7
Blood flows (mL/min):		
Liver	6.14	19.17
Fat	2.34	6.52
Muscle	3.81	11.13
Vessel-rich tissue	10.75	33.60
Bronchial lung area	1.79	5.514
Alveolar lung area	23.04	70.42
Volumes (mL):		
Liver	1.65	8.63
Fat	2.94	14.0
Muscle	19.09	162.7
Vessel-rich tissue	1.17	9.49
Bronchial lung area	0.2	1.29
Alveolar lung area	0.18	1.63
Partition coefficients [a]		
Blood:air	0.894	
Fat:blood	32.362	
Liver:blood	2.675	
Muscle:blood	1.871	
Kidney:blood	1.690	
Lung:blood	1.272	
Brain:blood	2.355	
Vessel rich:blood[b]	2.02	
Metabolic parameters		
$V_{max,total}$ ($\mu mol \cdot hr^{-1} \cdot kg^{-1}$)	465	200
$V_{max,liver}$ ($\mu mol \cdot hr^{-1} \cdot kg^{-1}$)	318	171
$V_{max,bronchial}$ ($\mu mol \cdot hr^{-1} \cdot kg^{-1}$)	77	13
$V_{max,alveolar}$ ($\mu mol \cdot hr^{-1} \cdot kg^{-1}$)	70	16
K_M (μM)	8	5

[a]Same for all species.
[b]Mean value of kidney:blood and brain:blood.

The Evelo et al. (1993) model suffers from a number of serious weaknesses. Several important parameters are not empirically derived. The partition coefficients are estimated theoretically, and the whole-body V_{max} and K_M are optimized. For the rat, this exercise generated a V_{max} value that was inconsistent with other in vivo data. Furthermore, sensitivity analyses revealed that the optimization was insensitive to variability in the value of K_M, so there is considerable uncertainty in the actual value of this parameter. The results pertaining to the relative importance of lung metabolism, however, are highly sensitive to the value of K_M. The separation of the lung into alveolar and bronchial areas and the "optimized" distribution of lung metabolism between the two areas also appear tenuous. Other limitations of the model are that metabolism is limited to the lung and the liver and that further metabolism of EB is not incorporated. In addition, the model was not adequately validated, and only limited sensitivity analyses are described. Finally, results for humans are discussed; however, the parameters used for the human model are not fully reported.

8.2.6. Medinsky et al. (1994)

The most recent PBPK model published for butadiene is the model of Medinsky et al. (1994) for 1,3-butadiene and EB uptake and metabolism in mice and rats. The Medinsky et al. model is a venous equilibration, flow-limited model with six physiological compartments**C**liver, lung, fat, slowly perfused tissue group, rapidly perfused tissue group, and blood**C**and a compartment representing the air in closed-chamber experiments. The model describes the oxidative metabolism of 1,3-butadiene in the liver and lung, as well as hydrolysis and glutathione conjugation of EB in the liver. In the mouse, hepatic oxidation of EB is also included. In addition to measuring actual partition coefficients, Medinsky et al. conducted closed-chamber experiments of 1,3-butadiene uptake with both mice and rats to test the predictions of their model.

Medinsky et al. (1994) measured partition coefficients for 1,3-butadiene and EB experimentally in vitro for both mouse and rat tissues. They found no significant differences between the two species, except for the muscle:air partition coefficient for 1,3-butadiene and the fat:air coefficient for EB (although the ultimate fat:blood coefficient was not significantly different). Organ and body weights were taken from specific experiments on 1,3-butadiene. The remaining physiological parameters were based on average literature values, with the exception of alveolar ventilation rate. Alveolar ventilation rates, conventionally defined as 70% of measured total ventilation rates, yielded overestimates of 1,3-butadiene uptake at low concentrations, consistent with observations by Johanson and Filser (1992) for other volatile organic chemicals. Therefore, "apparent" alveolar ventilation rates were obtained by

optimization to provide rates that yielded the best fit of the model to the EB uptake data. The optimized rates represented 63% of alveolar ventilation for both rats and mice.

Oxidation of 1,3-butadiene and EB (the latter in mouse liver only) and hydrolysis of EB were described using Michaelis-Menten kinetics. Glutathione conjugation of EB was assumed to be first order, based on the large K_M value reported by Csanády et al. (1992).

Rate constants for the metabolism of 1,3-butadiene and EB were taken from the in vitro data of Csanády et al. (1992). Apparent enzyme affinities (K_M) measured in vitro were used directly, whereas maximum metabolic rates (V_{max}) were scaled to the whole organs. However, when the organ microsomal concentrations reported by Csanády et al. are used to scale the metabolic rates similarly reported by Csanády et al., "[1,3-butadiene] uptake from the closed chamber is underestimated." Therefore, Medinsky et al. (1994) used literature values that were two to six times greater for microsomal concentrations in the liver and lung in order to successfully simulate the chamber study results. The parameter values used in the Medinsky et al. model are summarized in Table 8-5.

For validation of the model components pertaining to EB uptake and metabolism, model predictions were compared with the EB uptake data from the closed-chamber experiments of Filser and Bolt (1984) for rats and Kreiling et al. (1987) for mice, although these were the same data used to optimize the alveolar ventilation rates. The model predictions were deemed "adequate," although EB uptake was overestimated at the highest exposure concentration, especially for the rats (3,000 ppm). Medinsky et al. (1994) then compared model simulations of 1,3-butadiene uptake to their own closed-chamber data for mice and rats exposed to 1,3-butadiene and to data from the closed-chamber experiments of Bolt et al. (1984) for rats and Kreiling et al. (1986b) for mice and concluded that the model adequately predicted the in vivo uptake results. Medinsky et al. also compared model predictions with the 1,3-butadiene retention data of Bond et al. (1986) and found the results similar for exposure concentrations up to about 100 ppm. At higher concentrations, the model overestimated butadiene retention observed in mice. Furthermore, the blood concentrations of EB following 1,3-butadiene exposure, as reported by Bond et al. were overestimated by the model for both mice (except at the lowest exposure) and rats by about two- to fourfold, although Medinsky et al. suggested that the discrepancy might be attributable to EB loss from the blood during sampling.

No comprehensive sensitivity analysis for the model parameters was reported. Medinsky et al. (1994) did note that use of the microsomal concentrations reported by Csanády et al. (1992) resulted in underestimation of the 1,3-butadiene uptake from chamber studies. In addition, they investigated whether the model was sensitive to the different values obtained for the muscle:air

Table 8-5. Parameter values used in the Medinsky et al. (1993) PBPK model

Parameter	Rat	Mouse
Physiological parameters:		
Alveolar ventilation (L/hr/kg)[a]	17	41
Cardiac output (L/hr/kg)[b]	17	41
Body weight (kg)[c]	0.215-0.475	0.028-0.035
Blood flows (fraction of cardiac output):		
Liver	0.25	0.25
Fat	0.09	0.09
Lung	1.0	1.0
Slowly perfused tissues	0.15	0.15
Rapidly perfused tissues	0.51	0.51
Organ volumes (fraction of body weight):		
Liver	0.05	0.0624
Fat	0.09	0.10
Lung	0.0053	0.005
Slowly perfused tissues	0.71	0.70
Rapidly perfused tissues	0.0347	0.0226
Partition coefficients for 1,3-butadiene:		
Blood:air	1.49	1.34
Liver:blood	0.799	1.01
Lung:blood	0.617	1.10
Muscle:blood	0.987	2.99
Fat:blood	14.9	14.3
Partition coefficients for EB:		
Blood:air	50.4	36.6
Liver:blood	1.43	1.15
Lung:blood	1.09	1.54
Muscle:blood	0.393	0.645
Fat:blood	2.74	2.49
Tissue concentrations		
Liver microsomal concentration (mg/g liver)	35	35
Lung microsomal concentration (mg/g lung)	20	20
Liver cytosolic concentration (mg/g liver)[d]	108	82.8

Table 8-5. Parameter values used in the Medinsky et al. (1993) PBPK model (continued)

Parameter	Rat	Mouse
Rate constants for oxidative metabolism of 1,3-butadiene [d]		
Liver V_{max} (µmol/kg/hr)	62	338
K_M (µmol/L)	3.75	2.00
Lung V_{max} (µmol/kg/hr)		
K_M (µmol/L)	1.01	21.6
	7.75	5.01
Rate constants for EB metabolism in the liver [d]		
Oxidation V_{max} (µmol/kg/hr)		26
K_M (µmol/L)		15.6
Hydrolysis V_{max} (µmol/kg/hr)	260	754
K_M (µmol/L)	260	1590
glutathione conjugation K (L/kg/hr)	5.66	4.36

[a]Obtained by optimization.
[b]Ventilation/perfusion = 1.
[c]Depending on experiment simulated.
[d]From Csanády et al. (1992), with V_{max} values scaled to whole organ using above microsomal concentrations.

EB: 1,2-epoxy-3-butene.

partition coefficients for the mouse and rat and determined that the species-specific coefficients provided the best fits to their 1,3-butadiene uptake results for the two species. Medinsky et al. also determined that the inclusion of lung metabolism improves the model fit for the mouse, especially at lower exposure concentrations, but has little affect for the rat.

Based on their model simulations, Medinsky et al. (1994) suggested that lung metabolism may play an important role in 1,3-butadiene uptake and carcinogenesis. Their model predicts locally generated concentrations of EB that are 15 times greater in the mouse lung than in the rat lung, for a 6-h exposure to 10 ppm. Medinsky et al. recommended that more research be done to characterize 1,3-butadiene metabolism and target cells in the mouse lung and to understand the pharmacokinetics of DEB in different species. They further claimed that "quantitation of the concentrations of [1,3-butadiene], [EB], and [DEB] in target and non-target tissues of rats and mice after exposure to [1,3-butadiene] is essential for validation of existing models before these models can be applied to predict behavior in humans."

One of the major strengths of the Medinsky et al. (1994) model is that they experimentally measured partition coefficients and confirmed the results of Johanson and Filser (1993), suggesting that the empirical values for the partition coefficients for 1,3-butadiene differ significantly from the theoretical values used in previous models. Medinsky et al. also conducted closed-chamber experiments to obtain validation data for their model and investigated the role of lung metabolism in 1,3-butadiene uptake. Some limitations of the model include the fact that metabolism was restricted to the liver and lung, although other tissues are known to metabolize 1,3-butadiene as well (Chapter 3). In addition, the alveolar ventilation rates were determined by fitting experimental closed-chamber data, and there are uncertainties about the actual values for organ microsomal contents. Finally, only 1,3-butadiene oxidation was described in the lung, although rate constants for further metabolism of EB are also available from Csanády et al. (1992).

8.3. SUMMARY

Pharmacokinetic modeling of 1,3-butadiene has not yet elucidated the reasons for the interspecies differences in carcinogenic response between mice and rats. It appears that either the PBPK models are not sufficiently sophisticated to adequately model the relevant pharmacokinetics (e.g., the models may need to incorporate the production and disposition of DEB) or a pharmacodynamic component(s) (e.g., DNA susceptibility or repair) is required to accurately correlate dose to response.

Furthermore, uncertainties in the existing PBPK models and data make them unreliable for use in risk assessment. Serious uncertainties exist pertaining to the model structures, parameter values, and validation. For example, there are discrepancies among the models and

data as to the importance of extrahepatic and extrapulmonary metabolism, competitive interaction between 1,3-butadiene and EB for oxidative metabolism, and glutathione depletion, and none of the models fully describe the kinetics for DEB.

With respect to the parameter values, there are disagreements about the ventilation rate, which is a key parameter for determining 1,3-butadiene delivery, and about metabolic parameters. For example, measurements of V_{max} and K_M for the oxidation of 1,3-butadiene to EB in mouse, rat, and human liver microsomes by Csanády et al. (1992) and by Duescher and Elfarra (1994) differ by up to 80-fold, and Seaton et al. (1995) measured reaction rates for the oxidation of EB to DEB by rat and human liver microsomes that Csanády et al. were unable to detect (Chapter 3). Use of the in vitro metabolic data of Csanády et al. (1992) in the 1,3-butadiene PBPK models appears to result in an underprediction of total metabolism. Such underprediction could result from (1) an inability of the in vitro data to reflect the in vivo metabolic potency, (2) inaccuracies in the measurement of metabolic reaction rates or microsomal protein content in the tissues, or (3) a deficiency in the models such that they do not fully characterize 1,3-butadiene metabolism (e.g., by not including metabolism in other tissues). This is a critical issue for any PBPK-based extrapolation of carcinogenic risk from rodents to humans because there are no appropriate human in vivo PBPK data for 1,3-butadiene and thus interspecies extrapolation must rely on in vitro data or allometric scaling. There is also a paucity of human in vitro data for extension of the PBPK models to humans. The few measurements that have been made on a few metabolic parameters show a high amount of variability.

Another area of uncertainty is that of model validation. The existing models have been subjected to a very limited validation, mostly by comparison of simulation results with chamber uptake data. Virtually all of the model reports claim that the existing models adequately fit the validation data, despite important differences among the models. In some cases, this is not surprising because some of the model parameters have been determined by optimization against data similar to those being used for validation. In other cases, it suggests that the chamber data are relatively insensitive to various features of the models and might be of limited use for model validation. For the PBPK models to be more reliable, they should be validated against tissue concentration data for various metabolites in various tissues. More recently, these data have become available (Chapter 3), although they must be interpreted with caution because it appears that metabolites in some of the tissues are subject to further metabolism during the lag time between the termination of exposure and the measurement of tissue concentrations. The results of simulations using the Medinsky et al. (1994) model suggest that the model does not conform adequately to the tissue concentration data. Any PBPK model for 1,3-butadiene would require more rigorous validation before it could be considered reliable for use in risk assessment.

8.4. CONCLUSIONS

As discussed above, the existing PBPK models and data cannot explain the interspecies differences in 1,3-butadiene carcinogenicity. Uncertainties in the model structures and parameter values also prohibit their use in refining risk assessment dosimetry at this time. Some areas in which more research is needed include (1) evaluation of the kinetics of DEB in rodents as well as in humans, (2) investigation of the validity of the in vitro metabolic data for extrapolating to in vivo exposure, (3) clarification of the values of various physiological parameters such as the ventilation rate, (4) better characterization of the distribution of values for the human metabolic rates, and (5) more measurement of tissue concentrations of metabolites for model validation. It is possible that more information on the specific mechanisms of action is required to explain interspecies differences in the various target tissues.

In any event, the existing PBPK models and data are inadequate for developing a reliable alternative to the default methodology of using exposure to the parent compound as a dose surrogate for extrapolation of the carcinogenic risk from animals to humans. Any attempt to extrapolate the risk in rodents to humans, given the dramatic and unresolved interspecies differences between the mouse and rat, would involve far greater uncertainties than basing a risk assessment on the occupational data of Delzell et al. (Chapter 7). Ideally, a reliable, well-validated PBPK model with parameter values for humans could also be applied to analyzing different human exposure scenarios (e.g., extrapolating from occupational to environmental exposures). However, there are too many uncertainties in the PBPK modeling for that to be practicable at this time.